HFE Gene and Hereditary Hemochromatosis: A HuGE Review

E. H. Hanson[1], G. Imperatore[2], W. Burke[3]

Abbreviations: AF, attributable fraction; CI, confidence interval; HLA, human leukocyte antigen; HHC, hereditary hemochromatosis; OR, odds ratio; PCR, polymerase chain reaction; PPV, positive predictive value; TS, transferrin saturation; SF, serum ferritin

Authors' affiliations:

[1] United States Air Force School of Aerospace Medicine, Brooks Air Force Base, San Antonio, Texas

[2] Division of Diabetes Translation, Centers for Disease Control and Prevention, Atlanta, Georgia

[3] Departments of Medical History and Ethics and Medicine, University of Washington, Seattle, Washington

Reprint requests to Eric H. Hanson MD, MPH, 192 Oakwell Farms Parkway, San Antonio, TX 78218, USA

Running head:
HFE Gene and Hereditary Hemochromatosis

Abstract

Hereditary hemochromatosis (HHC) is an autosomal recessive disorder of iron metabolism characterized by increased iron absorption and deposition in the liver, pancreas, heart, joints, and pituitary gland. Without treatment, death may occur from cirrhosis, primary liver cancer, diabetes, or cardiomyopathy. In 1996, *HFE*, the gene for HHC, was mapped on the short arm of chromosome 6 (6p21.3). Two of the 37 allelic variants of the *HFE* gene described to date (C282Y and H63D) are significantly correlated with HHC. Homozygosity for the C282Y mutation is present in 52-100% of clinically diagnosed probands. Five percent of HHC probands are compound heterozygotes (C282Y/H63D), and 1.5% are homozygous for the H63D mutation; 3.6% are C282Y heterozygotes, and 5.2% are H63D heterozygotes. In 7% of cases, C282Y and H63D mutations are not present. In the general population, the frequency of the C282Y/C282Y genotype is 0.4%. C282Y heterozygosity ranges from 9.2% in Europeans to nil in the Asian, Indian subcontinent, African/Middle Eastern, and Australasian populations. The H63D carrier frequency is 22% in European populations. Accurate data on the penetrance of the different *HFE* genotypes are not available. Extrapolating from limited clinical observations in screening studies, an estimated 40-70% of persons with the C282Y homozygous genotype will develop biochemical evidence of iron overload. A smaller proportion will die from complications of iron overload. To date, population screening for HHC is not recommended because of uncertainties about optimal screening strategies, optimal care for susceptible individuals, laboratory standardization and the potential for possible stigmatization or discrimination.

epidemiology, genetics, hereditary hemochromatosis, hereditary haemochromatosis, HLA-H gene, *HFE* gene, iron overload

GENE

After two decades of intensive research the genetic complexity of hereditary hemochromatosis (HHC) is still unfolding. More than 20 years ago, HHC was described as an autosomal recessive disorder associated with the human leukocyte antigen (HLA)-A3 complex (1). Subsequently, HHC was linked to HLA-A on the short arm of chromosome 6 (2, 3). In 1996, Feder et al. (4) identified a 250-kilobase region located more than 3 megabases telomeric from the major histocompatibility complex on chromosome 6 that was identical by descent in 85 percent of HHC patients. In this region, they identified a gene related to the major histocompatibility complex class I family that they called HLA-H, but was subsequently named *HFE* (5). Feder et al. (4) described two missense mutations of this gene (C282Y and H63D) that accounted for 88 percent of the 178 HHC probands in their study. The *HFE* gene is located at 6p21.3- approximately 4.6 megabases telomeric from HLA-A, and covers approximately 10 kilobases(6).

The *HFE* protein is a 343 residue type I transmembrane protein that associates with class I light chain beta$_2$-microglobulin (4). The *HFE* protein product binds to the transferrin receptor and reduces its affinity for iron-loaded transferrin by 5- to 10-fold (7). The C282Y mutation alters the *HFE* protein structure and beta$_2$-microglobulin association, disrupting its transport to and presentation on the cell surface (8). The H63D mutation, in contrast, does not appear to prevent beta$_2$-microglobulin association or cell surface expression (9), indicating that the C282Y mutation results in a greater loss of protein function than does H63D (10). The localization of the *HFE* protein in the crypt cells of the duodenum (the site of dietary iron absorption) and its association with transferrin receptor in those cells are consistent with a role in regulating iron absorption (9, 11). The observation that *HFE*-deficient mice

(*HFE* gene knockout model) develop iron overload similar to that seen in human HHC provides evidence that the *HFE* protein is involved in regulating iron homeostasis (12).

GENE VARIANTS

To date, 37 allelic variants of the *HFE* gene have been reported (13), but this review will focus on the C282Y and H63D mutations (4). The C282Y mutation results from a G-to-A transition at nucleotide 845 of the *HFE* gene (845G→A) that produces a substitution of cysteine for a tyrosine at amino acid position 282 in the protein product. In the H63D mutation, a G replaces C at nucleotide 187 of the gene (187C→G), causing aspartate to substitute for histidine at amino acid position 63 in the *HFE* protein. In addition to C282Y and H63D, nine other missense mutations causing amino acid substitutions have been documented. In one, a substitution of a cysteine for serine at amino acid position 65 (S65C) has been implicated in a mild form of HHC (14). A number of intronic polymorphisms have also been found (13). One polymorphism occurs within the intron 4 (5569G-A) of the *HFE* gene in the binding region of the primer originally described by Feder et al. (4). One laboratory reported that when a polymerase chain reaction (PCR)-based restriction endonuclease digestion assay is used, the presence of this polymorphism might cause C282Y heterozygosity to be misdiagnosed as C282Y homozygosity (15, 16). However, three groups could not replicate this finding (17-19). In these studies, previously identified homozygotes for the C282Y mutation were confirmed by sequencing or using a primer external to the 5569G-A polymorphism, suggesting that genotyping errors due to this polymorphism are likely to be rare.

POPULATION FREQUENCIES

A computerized search of the PubMed database (National Library of Medicine) using the terms "hemochromatosis", "haemochromatosis", “HLA-H”, and "*HFE*" for papers published in English through February 2000 was performed. Of the studies identified, we included only those with sample sizes greater than 50 persons and where genotyping was performed for both the C282Y and H63D mutations in all individuals.

In table 1 the frequency of the *HFE* genotypes in clinically diagnosed probands is reported by geographic location. Except for two studies (14, 20), case definitions of HHC included diagnostic evidence of iron overload by liver biopsy or quantitative phlebotomy. In European countries, the estimated prevalence of homozygosity for the C282Y mutation in 2,229 HHC probands ranged from 52 percent (21) to 96 percent (22). In North America, C282Y homozygosity was present in 81 percent of 588 probands (range: 67 to 95 percent). Worldwide, among 2,929 probands 6.9 percent (95 percent CI: 6.0, 7.9) were homozygous for the wild allele. This finding suggests that nongenetic influence; additional HFE mutations; genetic redundancy, which is known to occur within the HLA region (23); or variation in additional genes affecting iron metabolism, as a recent twin study has suggested (24) may also cause iron overload. Heterozygosity for the H63D mutation and compound heterozygosity each accounted for 6 percent of European cases and 4 percent of cases in North America. Globally, 3.6 percent (95 percent CI: 2.9, 4.3) of proband patients had the C282Y/wild genotype, and 1.5 percent (95 percent CI: 1.1, 2.1) had the H63D/H63D genotype.

The estimated frequency of the *HFE* genotype in the general population is shown in table 2; 27 studies were evaluated. A total of 6,203 samples from European countries revealed on average a C282Y homozygous and heterozygous prevalence of 0.4 percent and 9.2 percent, respectively. However, C282Y homozygosity has not been reported in the general population of Southern or Eastern

Europe. The frequency of the C282Y heterozygosity is 1 to 3 percent in Southern and Eastern Europe and as high as 24.8 percent in Ireland. In North America (3,752 samples) these percentages were 0.5 percent (C282Y homozygous) and 9.0 percent (C282Y heterozygous). In the Asian, Indian subcontinent, African/Middle Eastern, and Australasian populations, C282Y homozygotes were not found and the frequency of C282Y heterozygosity was very low (range: 0 to 0.5 percent). C282Y/H63D compound and H63D homozygosity each accounted for 2 percent of the European general population and 2.5 percent and 2.1 percent in the American populations, respectively. The carrier frequency of the H63D mutation was 22 percent in Europe and 23 percent in North America.

Assuming that the proband studies correctly indicate that 78 percent of affected individuals are homozygous for C282Y, the estimated prevalence of HHC ranges from 51 to 64/10,000. In population-based intervention trials, the estimated prevalence of homozygosity based on phenotype, defined as biochemical evidence of iron overload, is 50 per 10,000 (25). In primary care settings among whites, the estimated prevalence of clinically proven or biopsy proven HHC is 54 per 10,000 (26). Higher prevalence (80/10,000) was obtained in one study when elevated transferrin saturation (TS) alone was used for a case definition (27). This may simply reflect the fact that a significant proportion of unaffected or heterozygous individuals have TS levels above the cutoff, especially when TS thresholds of 50% are used (25).

DISEASE

HHC is a disorder of iron metabolism characterized by increased iron absorption. Iron is progressively deposited in various tissues, particularly the liver, pancreas, heart, joints, and pituitary gland. Beside HHC, there are other genetic causes of iron overload. For example, families have been

identified with a clinical syndrome indistinguishable from HHC but without linkage to chromosome 6 (28, 29). Recently, linkage to the *Tfr2* gene was described in two such families (30). In addition, a rare autosomal condition, juvenile hemochromatosis that results in rapid accumulation of iron, has recently been mapped to chromosome 1 (31). Two other syndromes, neonatal hemochromatosis and African iron overload syndrome, have been described, but genetic influences or other contributing factors are not well understood.

Many studies have evaluated the occurrence of clinical symptoms in persons with HHC, but differences in sample selection, population characteristics, and case definition make it difficult to characterize attributable morbidity. Studies in clinical settings may overestimate the disease burden because patients with advanced disease are more likely to be included. Conversely, studies including "healthy people" (e.g., blood donors, asymptomatic adults in a screening program) may, by excluding sick persons, underestimate the disease burden. These limitations highlight the need for controlled, population-based studies that allow unbiased ascertainment of HHC's impact, including the extent to which its common manifestations are etiologically related. In the absence of such studies, current knowledge about clinical manifestations is based largely on observation of patients seen in referral centers.

Phenotypic expression of HHC, which is variable, appears to depend on a complex interplay of the severity of the genetic defect, age, sex, and such environmental influences as dietary iron, the extent of iron losses from other processes, and the presence of other diseases or toxins (e.g., alcohol) (32). The rate of iron accumulation and the frequency and severity of clinical symptoms vary markedly; early complaints may include fatigue, weakness, joint pain, palpitations, and abdominal pain (33). Because these symptoms are relatively nonspecific, HHC is often not diagnosed at this stage. The disease can

ultimately lead to hyperpigmentation of the skin, arthritis, cirrhosis, diabetes mellitus, chronic abdominal pain, severe fatigue, hypopituitarism, hypogonadism, cardiomyopathy, primary liver cancer, or an increased risk of certain bacterial infections (34). Most of these advanced complications are also common primary disorders, and iron overload can be missed at this stage unless looked for specifically.

The liver is usually the first organ to be affected and hepatomegaly is one of the most frequent findings at clinical presentation (35). In one study, noncirrhotic probands at clinical presentation reported weakness, lethargy, and loss of libido more frequently than probands with cirrhosis, but symptoms of abdominal pain were markedly more frequent in the cirrhotic patients (34). The proportion of patients with cirrhosis at clinical presentation has varied from 22 to 60 percent (34, 36, 37).

Primary hepatocellular carcinoma is 200 times more common in HHC patients (34) but it rarely occurs without cirrhosis. Hepatocellular carcinoma has been reported to account for 30 to 45 percent of deaths among the HHC patients seen in referral centers (38, 39). In patients with this kind of cancer, the prevalence of HHC ranges from 11 to 15 percent (40).

Diabetes mellitus is the major endocrine disorder associated with HHC. The mechanisms responsible are still obscure, but iron deposition that damages the pancreatic beta cells and insulin resistance (38) have been postulated. Hypogonadism also occurs and is caused primarily by a gonadotropin deficiency resulting from iron deposition at the pituitary or hypothalamic levels. Other endocrine disorders involving an effect of HHC on the thyroid, parathyroid, or adrenal glands are rarely seen.

Cardiac manifestations include cardiomyopathy and arrhythmias. Congestive heart failure has been seen in 2 to 35 percent and arrhythmias are present in 7 to 36 percent of HHC patients (41).

Increases in melanin (42) lead to hyperpigmentation in 27 to 85 percent of patients (41). Loss of body hair, atrophy of the skin and koilonychia (dystrophy of the fingernails) may also occur.

Arthropathies are found in 40 to75 percent of patients (38) and may affect the second and third metacarpal phalanges (43), wrist, shoulder, knees, or feet.

Symptoms of HHC usually appear between ages 40 and 60 years with the onset normally later in women (44). This difference may relate to loss of iron with menstruation, pregnancy and lactation and to their lower iron intake relative to their iron needs (45). Men are more likely to develop clinical disease. Presenting signs and symptoms of HHC also vary by sex, with women more likely to present with fatigue, arthralgia, and pigmentation changes and men presenting more often with symptoms of liver disease (37).

Symptoms and disease complications increase with age; in one study, 73 percent of men and 44 percent of women HHC homozygotes over age 40 had at least one clinical finding consistent with HHC (25). A smaller proportion, not yet well defined, is likely to develop potentially life-threatening complications (21, 46-48).

Treatment

Periodic phlebotomy or venesection to remove iron is a safe, inexpensive, and effective treatment for HHC. Venesection is usually initiated when serum ferritin (SF) concentrations indicate excess accumulation of iron stores. For example, the College of American Pathologists recommends initiation of venesection when SF reaches 300µg/L in men and 200µg/L in women (41); however, the appropriate SF cutoff for women may vary with their reproductive status (49). There have been no controlled trials of phlebotomy treatment, but observational studies in referral centers suggest that iron removal markedly increases survival (34, 38, 50, 51). Dietary management includes avoidance of iron

supplements, excess vitamin C, and uncooked seafood, which is known to increase the risk of *Vibrio vulnificus* and *Salmonella enteritidis* infections in HHC patients (49).

If treated early in the course of the illness, complications improve in some patients after iron depletion. In patients with established iron overload, liver function, weakness and lethargy (or fatigue), right upper quadrant abdominal pain, abnormal skin pigmentation, and cardiomyopathy usually improve, but hypogonadotropic hypogonadism does not (49). Response to treatment for patients with arthralgias is highly variable. Removal of excess iron does not reverse diabetes but can reduce insulin requirements (34, 50). Chelation therapy, which increases iron excretion, is less efficient and more expensive than phlebotomy. In general, management of HHC complications including liver failure, cardiac failure, and diabetes, differs little from the conventional management of these diseases.

Mortality

The prognosis of untreated HHC relates to the duration and amount of excess iron present at diagnosis. The severity of overload can be estimated from the number of venesections required to deplete iron stores (34, 36).

As liver iron concentration increases, the prevalence of diabetes, cirrhosis, and cardiac disease is higher (33) which has implications for mortality rates.

In general, persons with symptomatic HHC have lower survival than age- and sex-matched normal populations (34, 36) with the presence of cirrhosis being the primary factor affecting survival. However, studies from three referral centers indicate that if phlebotomy is initiated before cirrhosis develops, survival does not differ from that of the general population after adjustment for age and sex (34, 36, 39). Whether diabetes mellitus affects survival is controversial. Niederau et al. found reduced survival in HHC patients presenting with diabetes at the diagnosis (34), but 64 percent of those with

diabetes also had cirrhosis, and thus their increased mortality might have been due to that complication. Even so, in a multivariate analysis with diabetes, cirrhosis, arthritis, age, and sex as covariates, the presence of diabetes significantly increased mortality (relative risk of 4.3) (34). In contrast, in an analysis of a Canadian HHC cohort that controlled for cirrhosis, diabetes did not increase risk of death (36).

In evaluating these observations, the fact that data on the efficacy of treatment were derived from observational studies in referral centers rather than from population-based intervention trials should be considered, as should the limited availability of data on the efficacy of treating asymptomatic persons. The most widely quoted studies on the benefit of early treatment have been on the clinical outcome of a cohort of patients diagnosed at two clinical centers in Germany over a 40-year period (34, 38). In the most recent report, data were presented on 251 patients (89 percent male) followed for a mean of 14 years; 109 patients (43 percent) were noncirrhotic at diagnosis, 68 had other clinical findings and 41 were asymptomatic. The life expectancy of these patients as measured over the follow-up period, was indistinguishable from that of the general population, corrected for age and sex. Adams et al. reported similar data (36). While such reports are consistent with a significant benefit from phlebotomy treatment, they do not constitute proof that treatment of asymptomatic persons with HHC improves outcome, because the numbers are small and there was no untreated control group. An alternative explanation is that disease progression is minimal in many people with HHC genotypes who are asymptomatic or in the early stages of iron overload.

Available data on survival are based on clinical cohorts diagnosed primarily on the basis of symptoms, and thus they reflect outcome in relatively late manifestations of the disease rather than in people diagnosed early. Historically, HHC survival has been poor. Before the introduction of insulin in 1921 and then phlebotomy treatment in 1935, the time from symptomatic presentation to death was 18

months (52). In recent times, HHC patients had more deaths from hepatocellular carcinoma, cardiomyopathy, liver cirrhosis, and diabetes mellitus than would be expected in the general population (34). In 1992, the HHC-associated mortality rate in the American population was reported at 1.8 deaths per million (0.0002 percent) (53), far lower than the estimated prevalence of HHC, suggesting that HHC is underdiagnosed, that the penetrance of the disease is low, or both.

Genotype-phenotype correlation

Factors that limit the comparability of studies and estimates of disease risk include the heterogeneity of clinical presentations, limited population characteristics studied and the lack of a uniform case definition. A pooled analysis on 14 studies in whites, including 2,205 cases and 5,604 controls, showed the highest risk for iron overload was associated with homozygosity for the C282Y mutation (odds ratio (OR)= 4,383, 95 percent CI: 1,374 to > 10,000; attributable fraction (AF) = 0.73) (54). The pooled OR for iron overload among persons with compound heterozygosity for C282Y and H63D mutations was 32 (95 percent CI: 18.5 to 55.4; AF = 0.06). H63D homozygosity carried a lower risk (pooled OR = 5.7, 95 percent CI: 3.2 to 10.1; AF = 0.01). Heterozygosity for the C282Y mutation was associated with a four-fold risk of iron overload (OR = 4.1, 95 percent CI: 2.9 to 5.8; AF = 0.03), but for this genotype significant differences in the OR were detected across the studies, making this result of uncertain significance. A small association also existed between iron overload and heterozygosity for H63D mutation (OR = 1.6, 95 percent CI: 1.0 to 2.6; AF = 0.03). Potential biases, however, might have influenced the magnitude of these associations. First, investigators used a variety of diagnostic criteria. Second, ascertainment of both cases and controls varied markedly and often was not clearly defined. Lastly, inappropriate control populations and the lack of consideration of modifiers such

as age, sex, dietary iron intake, and alcohol consumption may have decreased the accuracy of estimates.

INTERACTIONS

The clinical expression of HHC is influenced by a variety of factors, both genetic and environmental. In *HFE* knockout mice, mutations of other genes involved in iron metabolism, such as beta$_2$-microglobulin, transferrin receptor, and DTM1 (transmembrane iron import molecule), strongly modify the amount of liver iron (55), suggesting that modifier genes may influence the course of HHC in humans. There is also evidence that sex plays a primary role in the clinical manifestation of HHC. Family studies based on HLA linkage report an equal frequency of affected brothers and sisters, as expected for an autosomal recessive disorder, but the proportion of females among probands diagnosed on the basis of clinical symptoms is 11 to 35 percent lower than in males (33, 34, 39). And in a large screening trial, the prevalence of iron overload as determined by liver biopsy or phlebotomy was twice as frequent in males as females (47). This sex difference has been attributed to the lower degree of iron overload in women because of menstruation, pregnancy and lactation.

The environment has also been reported to modify the expressivity, or penetrance, of the HHC genotype. Possible positive (beneficial) modifiers of disease phenotype include pregnancy and menstruation in females and chronic blood loss (gastrointestinal bleeding, regular hematuria, helminthic or other parasitic infections) and regular blood donation in both men and females. Detrimental factors include alcohol abuse, excessive iron intake, or other factors that increase iron stores (e.g., vitamin C). Tannates, phytates, oxalates, calcium and phosphates also modify HHC because they are known to bind iron and inhibit iron absorption (49).

Chronic viral hepatitis B and C and metals such as zinc and cobalt may also influence expression of HHC (49, 56). Iron modulates the course of hepatitis B (57), and iron reduction has been shown to decrease the severity of chronic hepatitis C while increasing the likelihood of response to antiviral therapy. Hepatitis C virus infection and *HFE* mutations have also been identified as risk factors for porphyria cutanea tarda (57).

Conte et al. (58), who studied 894 diabetic patients from Northern Italy, calculated an OR for HHC of 6.3 and a 1.34 percent prevalence of HHC in type II patients. The authors suggested that screening diabetic patients for HHC might be beneficial (58). However, Frayling et al. found a type II C282Y homozygosity prevalence of 0.42 percent, similar to an age-matched normoglycemic control group (59). Larger, population-based studies are needed to reach definitive conclusions.

Iron overload can be a complication of certain disorders that are characterized by increased erythropoietic activity. Studies evaluating the impact of HHC on hereditary spherocytosis and acquired anemia have been inconsistent (60).

LABORATORY TESTS

Transferrin saturation and serum ferritin

The most widely used biochemical markers of body iron status are transferrin saturation percent (TS = serum iron/total iron binding capacity x 100) and SF values. Elevated TS is usually present before symptoms occur or other studies indicate iron overload. The cutoff TS values recommended for screening have varied from 45 to 70 percent (26, 41, 61, 62). If the TS is above the threshold and no other explanations exist for iron overload (e.g., chronic anemias, liver diseases due to alcohol or viral

infection) the test should be repeated after an overnight fast (41). Subjects should avoid iron and vitamin C supplements for at least 24 hours before testing. Simultaneously, tests of liver function, and a complete blood count should be performed. A second elevated TS indicates that the person may have HHC. If SF levels are also elevated then additional diagnostic testing (quantitative phlebotomy or liver biopsy) is recommended to confirm the presence of iron overload (17). In persons identified as having iron overload related to HHC by this screening and diagnostic process, the probability of developing clinical complications is uncertain. Family and screening trials suggest that 50 to 70 percent of males and 40 to 50 percent of females will develop symptoms or complications of HHC (25, 63), but most complications recorded in such studies were common and nonspecific clinical manifestations such as joint pain and diabetes. In the absence of control groups, the proportion of complications attributable to HHC is difficult to determine; as a result, the probability of developing clinical complications may be considerably lower.

The analytical validity of the TS test can be evaluated by its sensitivity, specificity, and predictive value for the genotype, which depend in turn on the characteristics of the test and the underlying gene frequency. Using data from family studies and screening trials, Bradley et al. (25) found that screening at a TS cutoff value of 50 percent would identify approximately 94 percent of homozygote men and 82 percent of homozygote women and approximately 6 percent of men and 3 percent of women would be false positive results. Assuming an HHC genotype prevalence of 50 in 10,000, the odds of being affected given a positive result would be about 1:12 for males and 1:8 for females, corresponding to a positive predictive value (PPV) of 8 percent and 11 percent, respectively. PPVs using HLA typing as the standard increase when an initial elevated TS is followed by fasting TS higher than the first TS (64).

The lack of a standard case definition makes it difficult to assess the clinical validity of the TS test. In a large, population-based screening study, the sensitivity of a single elevated TS for HHC (defined as the presence of iron overload with SF ≥ 95th percentile and mobilizable iron >99th percentile) was 100 percent, with a specificity of 97 percent and a PPV of 8 percent (27). The PPV rises with increasing prevalence of HHC. In a screening study of patients with liver disease, who presumably are more likely to have HHC, the PPV of a single elevated TS test was 41 percent (65). In patients with diabetes, the PPV of repeated elevated TS tests ranged from 63 to 83 percent when HHC was defined as increased liver iron stores (58, 66, 67).

Ferritin is an intracellular iron storage protein and SF concentration significantly correlates with body iron stores (1ng/mL = 10 mg of stored iron) (68). SF values, but not TS values, are associated with HHC clinical signs, and SF is higher for those with clinical manifestations (25). SF has been used as a second screening test in many trials, and it can be very effective in reducing the number of false positives (47), if cutoffs appropriate for age and sex are used. Elevation of the SF concentration in HHC must be differentiated, however, from other liver disorders such as alcoholic liver disease, chronic viral hepatitis, and nonalcoholic steatohepatitis. Serum ferritin is also an acute phase reactant and can be elevated during infection, chronic inflammation, or when the subject has a histiocytic neoplasm (41).

HFE gene mutation analysis

Methodologies used for identifying the C282Y and H63D allelic variants of the *HFE* gene should be sensitive and specific; however, data on technical performance are pending. The accuracy of the mutation analysis in predicting the HHC phenotype is uncertain because of genetic heterogeneity, reduced penetrance, and the lack of a standardized HHC case definition. As C282Y and H63D account for most but not all clinically diagnosed cases of HHC in whites (table 1), it is plausible that

other mutations or other genes yet to be identified (30, 31) may also cause HHC. In addition, even in persons with detectable mutations the penetrance of the *HFE* genotype is not complete. In the general population, the C282Y/H63D and H63D/H63D genotypes occur more frequently than the C282Y/C282Y genotype (table 2), but among clinically diagnosed probands the C282Y/H63D and H63D/H63D account for only a small proportion of cases, suggesting low penetrance of the H63D allele (table 1). Studies on the *HFE* protein showing a lower loss of protein function with the H63D mutation corroborate this observation (7, 41). Reduced penetrance is likely for the C282Y/C282Y genotype, as indicated by case reports of elderly persons with this genotype and no evidence of significant disease (69, 70). That death from HHC complications does not lead to underrepresentation of this genotype in the elderly is suggested by a study of 600 patients over age 70 years that reported a prevalence of C2828Y homozygosity of 1 in 150 (71). Taken as a whole, this data indicates that mutation analysis alone cannot provide a simple positive or negative screening test for HHC.

POPULATION TESTING

In populations of European descent, the prevalence estimates for C282Y homozygosity is 4 per 1,000 and for C282Y heterozygosity is 90 per 1,000 (table 2). The estimated U.S. population in November 1999 was 273,866,000, with 196,409,000 (71.7 percent) white persons (non-Hispanic) (72). Using these figures, at least 1,095,464 white (non-Hispanic) persons are C282Y homozygous and another 24,647,940 are C282Y heterozygous carriers. This estimate of the potential HHC public health burden would be enlarged if other ethnic groups and other etiologies of primary and secondary iron overload disorders were included.

Although HHC meets most of the World Health Organization criteria for population screening, crucial questions remain to be answered before recommending population screening (17, 40, 73, 74). In particular, more information is needed about penetrance of clinical expression among persons with elevated TS or *HFE* mutations. Data about the disease burden associated with HHC is inadequate in the general population, and studies conducted in cohorts of patients with diseases associated with HHC are inconclusive in this regard (31, 58, 59, 75). Questions on screening accuracy, available diagnostic tests and the efficacy of early treatment need to be answered. Evidence on the efficacy of treatment is only available from retrospective studies and case series (34, 36, 50), and indicates that therapeutic phlebotomy improves survival among subjects with HHC who have clinical disease. For ethical reasons, randomized, controlled trials comparing therapeutic phlebotomy with no treatment cannot be undertaken and the effect of early phlebotomy treatment on life expectancy and quality of life cannot be objectively determined.

Genetic testing for HHC raises general concerns of stigmatization, discrimination, diminished self-worth, and as a result, concerns about possible breaches of privacy and confidentiality. In particular, there is concern about the possible loss of insurance coverage and employment, which has been reported in anecdotal cases (76). Efforts to pass laws prohibiting discrimination in health insurance and employment have not yet ensured full protection from such discrimination.

The cost-effectiveness of population-based TS screening has been assessed in numerous favorable but conservative studies (49, 62, 77, 78). Each study had important limitations however. First, they all assumed that people will fully follow the recommendations and that there will be no dropouts during the screening, diagnostic, and treatment process. Second, none included costs to individuals. More generally, cost-effectiveness analysis is very sensitive to variation in the proportion of individuals

who test positive and would eventually develop clinical manifestations of the disease in question (49, 62, 77, 78). It may be that for low levels of penetrance, screening for HHC is not cost-effective. This hypothesis, however, has not yet been tested. Another area that requires further study is consideration of the relative cost and efficacy of genetic testing versus phenotypic screening tests. An efficient method of screening after identification of a proband is testing spouses of probands with the results of the spouse's test guiding further testing of children (79, 80).

Additional issues in population-based testing for HHC include the need for a centralized management and coordination mechanism for outreach and information services. The Centers for Disease Control and Prevention and the National Human Genome Research Institute have started a process that facilitates organization, education of health care providers and patients, and cooperation of individuals with expertise in this unique disorder. Explorations of the contributions of the gene-gene and gene-environment interactions in HHC offer unique opportunities to create valuable models to guide future programs in genetic medicine and genetic epidemiology.

Acknowledgement

The authors thank Linda Bradley, Leslie O'Leary, Shelley Reyes, and Marta Gwinn for their assistance in completing this review.

APPENDIX TABLE. Internet sites pertaining to the HFE gene and hereditary hemochromatosis

Type of site	World Wide Web URL
Genetic Databases	
OMIM - Online Mendelian Inheritance in Man: gene entry for HHC	http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?235200
GDB Central Node listing for *HFE*	http://gdbwww.gdb.org/gdb-bin/genera/accno?GDB:119309
GenAtlas: genetic database for *HFE*	http://www.citi2.fr/cgi-bin/detgen?SYMB=*HFE*&DISD=0
GeneCards: human genes, proteins, and diseases	http://bioinfo.weizmann.ac.il/cards-bin/cardsearch.pl?search=*hemochromatosis*
UniGene—research information on HHC on National Center for Biotechnology Information search site	http://www.ncbi.nlm.nih.gov/UniGene/clust.cgi?ORG=Hs&CID=20019
The Human Mutation Database entry for *HFE* gene	http://www.uwcm.ac.uk/uwcm/mg/ns/1/119309.html
Educational Resources	
Centers for Disease Control and Prevention—At-A-Glance information on HHC	http://www.cdc.gov/nccdphp/dnpa/hemochromatosis.htm
National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health	http://www.niddk.nih.gov/health/hematol/pubs/hemoch/hemoc.htm
GeneClinics online medical textbook with chapters on inherited disorders	http://www.geneclinics.org
GeneTests- directory of research and clinical laboratories performing genetic tests	http://www.genetests.org
Support Groups	
American Liver Foundation	http://www.liverfoundation.org
Iron Disorders Institute, Inc	http://www.irondisorders.org
International links to HHC support groups	http://members.tripod.com/~hemochromatose/linkseng.html
National Organization for Rare Disorders (NORD)—consumer information, resources and advocacy, HHC information	http://www/stepstn.com/nord/rdb_sum/13.htm

TABLE 1. *HFE* genotype frequencies in clinically diagnosed probands

Study Population	Genotype							
Europe	**C282Y/Wild Frequency (%) (95% CI)**	**C282Y/C282Y Frequency (%) (95% CI)**	**H63D/Wild Frequency (%) (95% CI)**	**H63D/H63D Frequency (%) (95% CI)**	**C282Y/H63D Frequency (%) (95% CI)**	**Wild/Wild Frequency (%) (95% CI)**	**Subjects**	**References**
France	0.9 (0.1, 3.3)	96.3 (92.9, 98.4)	0.5 (0.0, 2.5)	0.5 (0.0, 2.5)	1.8 (0.5, 4.7)	0	N=217; unrelated probands meeting the following criteria: 1) elevated TS (>45%), and 2) histological features of hemochromatosis at liver biopsy, and 3) iron hepatic index ≥1.9; and /or 4) histological hepatic iron index ≥0.19; and/or 5) excess iron (M>5g, F>3g) removed by phlebotomy	Brissot 1999 (22)
France	1.0 (0.0, 5.5)	81.8 (72.8, 88.9)	3.0 (0.6, 8.6)	4.0 (1.1, 10.0)	7.1 (2.9, 14.0)	3.0 (0.6, 8.6)	N=99; unrelated patients with clinical diagnosis of hemochromatosis	Martinez 1997 (81)
France	4.3 (1.2, 10.5)	72.3 (62.2, 81.1)	8.5 (3.8, 16.1)	2.1 (0.3, 17.5)	4.3 (1.2, 10.5)	8.5 (3.8, 16.1)	N=94; unrelated probands diagnosed by iron indices, liver biopsy, or response to phlebotomy	Borot 1997 (82)
France (Brittany)	6.4 (4.5, 8.8)	52.4 (48.0, 56.7)	13.4 (10.6, 16.6)	3.0 (1.7, 4.9)	9.6 (7.2, 12.4	15.2 (12.3, 18.6)	N=531; unrelated probands with histological total iron score >3, liver iron >36μmol/g, or hepatic iron index >2,or excess iron (M>5g, F>3g) removed by phlebotomy	Moirand 1999 (21)
France (Brittany)	4.4 (3.0, 6.1)	80.2 (77.1, 83.0)	3.8 (2.5, 5.5)	1.1 (05, 2.2)	5.6 (4.1, 7.6)	4.9 (3.5, 6.8)	N=711; unrelated probands meeting two or more of the following: 1) elevated TS (M>60%, F>50%), 2) elevated serum ferritin (M >400μg/L, F >300μg/L), 3) serum iron >20μmol/L	Mura 1999 (14)
Germany	0	89.5 (78.5, 96.0)	5.2 (1.1, 14.6)	0	3.5 (0.4, 12.1)	1.8 (0.0, 1.4)	N=57; unrelated probands meeting one or more of the following criteria: hepatic iron concentration >33μmol/g, hepatic iron index >2, or elevated mobilizable iron by quantitative phlebotomy; M=45 and F=12	Gottschalk 1998 (83)
Ireland	2.6 (0.3, 9.0)	89.7 (80.8, 95.5)	1.3 (0.0, 6.9)	0	3.9 (0.8, 10.8)	2.6 (0.3, 9.0)	N=78; unrelated probands with clinical history, persistent elevated iron indices, 60 probands out of 78 had hepatic iron staining of >3+	Ryan 1998 (84)
Italy	5.9 (3.0, 10.2)	64.5 (56.5, 70.7)	8.6 (4.9, 13.5)	1.6 (0.0, 4.6)	5.4 (2.6, 9.6)	14.0 (9.2, 19.6)	N=186; unrelated probands meeting the following criteria: 1) repeated TS >50% and elevated serum ferritin, 2) hepatic iron staining of 3+ or 4+, 3) hepatic iron index ≥2 or excess iron removed by phlebotomy (M>5g, F>3g), 4) no iron loading anemia or history of blood transfusions; M=162, F=26	Piperno 1998 (85)
Northeast Scotland	0	90.7 (79.7, 96.9)	0	0	5.6 (1.2, 15.4)	3.7 (0.0, 12.7)	N=54; unrelated probands diagnosed by clinical features, iron indices, or liver biopsy	Miedzybrod-ska 1999 (86)
Sweden	1.1 (0.0, 6.2)	92.0 (84.1, 96.7)	1.1 (0.0, 6.2)	1.1 (0.0, 6.2)	3.5 (0.7, 9.8)	1.1 (0.0, 6.2)	N=87; unrelated probands with elevated TS (M>60%, F>50%) and elevated SF (>300mg/dL) or liver biopsy with increased iron staining; M=67, F=26; mean age M= 47 and F= 49	Cardoso 1998 (87)
United Kingdom	0.9 (0.0, 4.8)	91.3 (84.6, 95.8)	0	0.9 (0.0, 4.8)	2.6 (0.5, 7.4)	4.3 (1.4, 9.9)	N=115; probands receiving care at four UK medical centers, diagnosed by hepatic index >1.9 or by >5g total iron removed by phlebotomy	Robson 1997 (88)

Total	**3.9 (3.1, 4.8)**	**75.4 (73.6, 77.2)**	**5.9 (4.9, 6.9)**	**1.6 (1.1, 2.2)**	**5.8 (4.9, 6.9)**	**7.4 (6.3, 8.5)**	**Europe total number of probands=2,229**

Table continues

TABLE 1. *HFE* genotype frequencies in clinically diagnosed probands (continued)

North America	Genotypes							References
	C282Y/Wild	C282Y/C282Y	H63D/Wild	H63D/H63D	C282Y/H63D	Wild/Wild	Subjects	
Canada (Ontario)	0	95.3 (90.1, 98.3)	1.6 (0.2, 5.5)	0	0	3.1 (0.9, 7.8)	N=128; probands receiving care at a tertiary medical center, diagnosed by hepatic index >1.9g or by >5g total iron removed by phlebotomy	Adams 1998 (69)
United States (Alabama)	14.9 (7.7, 25.0)	59.5 (47.4, 70.7)	8.1 (3.0, 16.8)	4.1 (0.8, 11.4)	5.4 (1.5, 13.3)	8.1 (3.0, 16.8)	N=74; unrelated white probands found during medical care delivery to have an elevated TS (TS M≥60% and F≥50%)	Barton 1997 (20)
United States (New York)	6.6 (1.8, 15.9)	67.2 (54.0, 78.7)	4.9 (1.0, 13.7)	4.9 (1.0, 13.7)	8.2 (2.7, 18.1)	8.2 (2.7, 18.1)	N=61; Probands diagnosed by liver biopsy or iron indices (elevated TS and SF) with no known disorders associated with iron overload	Sham 1997 (89)
United States	1.4 (0.2, 4.8)	82.3 (76.8, 88.3)	2.7 (0.7, 6.8)	1.4 (0.2, 4.8)	5.4 (2.4, 10.4)	6.8 (3.3, 12.2)	N=147; unrelated probands of European origin diagnosed by serum iron measures, liver biopsy or response to phlebotomy	Beutler 1996 (90)
United States	0.6 (0.0, 3.1)	83.1 (76.8, 88.3)	3.9 (1.6, 7.9)	0.6 (0.0, 3.1)	4.5 (2.0, 8.7)	7.3 (4.0, 12.2)	N=178; probands meeting two or more of the following criteria: hepatic iron concentration >4500µg/g, hepatic iron index >2, grade 3+ or 4+ stainable iron in liver, >4g total iron removed by phlebotomy	Feder 1996 (4)
N. America Total	**3.1 (1.8, 4.8)**	**81.0 (77.5, 84.1)**	**3.7 (2.4, 5.6)**	**1.5 (0.7, 2.9)**	**4.3 (2.8, 6.2)**	**6.4 (4.6, 8.8)**	**North America total number of probands=588**	
Australia	0	100 (97.0, 100)	0	0	0	0	N=112; persons from 26 families with HH meeting at least two of the following criteria: hepatic iron concentration of 80µM/g, hepatic iron ≥1.9g or >5g total iron removed by phlebotomy	Jazwinska 1996 (91)
Global Total	**3.6 (2.9, 4.3)**	**77.5 (75.9, 79.0)**	**5.2 (4.4, 6.1)**	**1.5 (1.1, 2.1)**	**5.3 (4.5, 6.2)**	**6.9 (6.0, 7.9)**	**Global total number of probands=2,929**	

TABLE 2. *HFE* genotype frequencies in the general population

Study Population	Genotype							
Europe	**C282Y/Wild Frequency (%) (95% CI)**	**C282Y/C282Y Frequency (%) (95% CI)**	**H63D/Wild Frequency (%) (95% CI)**	**H63D/H63D Frequency (%) (95% CI)**	**C282Y/H63D Frequency (%) (95% CI)**	**Wild/Wild Frequency (%) (95% CI)**	**Subjects**	**References**
Denmark	11.0 (7.0, 16.2)	0	20.0 (14.7, 26.2)	1.5 (0.3, 4.3)	2.5 (0.8, 5.7)	65.0 (58.0, 71.6)	N=200; unrelated Danish blood donors	Steffensen 1998 (92)
Denmark	13.7 (9.4, 19.0)	1.4 (0.3, 4.0)	12.3 (8.3, 17.4)	0	0	72.6 (66.2, 78.4)	N=219; dried blood spots from neonatal screening programs	Merryweather-Clarke 1999 (93)
Faeroes	6.4 (3.4, 10.9)	1.1 (0.1, 3.8)	18.2 (12.9, 24.5)	3.2 (1.2, 6.9)	1.6 (0.3, 4.6)	69.5 (62.4, 76.0)	N=187; dried blood spots from neonatal screening programs	Merryweather-Clarke 1999 (93)
France	8.4 (3.7, 15.9)	0	23.2 (15.1, 32.9)	4.2 (1.2, 10.4)	0	64.2 (53.7, 73.8)	N=95; “healthy unrelated controls”	Borot 1997 (82)
France (Brittany)	3.6 (1.1, 8.2)	0	23.7 (16.9, 31.7)	3.6 (1.2, 8.2)	2.2 (0.4, 6.2)	66.9 (58.4, 74.5)	N=139; “randomly selected from the general population”	Moirand 1999 (21)
France (Brittany)	12.2 (9.2, 15.8)	0.5 (0.1, 1.8)	24.4 (20.3, 28.8)	0.7 (0.2, 2.1)	2.2 (1.0, 4.1)	60.0 (55.1, 64.8)	N=410; “unrelated randomly selected as controls”	Mura 1999 (21)
France (West Brittany)	13.8 (9.8, 18.6)	0.8 (0.1, 2.8)	25.6 (20.3, 31.4)	2.4 (0.9, 5.1)	3.5 (1.6, 6.6)	53.9 (47.6, 60.2)	N=254; “healthy persons from a western region of France (Finistere Sud)”	Jezequel 1998 (94)
Germany	3.3 (1.1, 7.5)	0	19.0 (13.1, 26.1)	2.0 (0.4, 5.6)	2.0 (0.4, 5.6)	73.9 (66.2, 80.6)	N=153; healthy blood donors	Gottschalk 1998 (94)
Germany	7.0 (3.1, 13.3)	0	21.7 (14.6, 30.4)	3.5 (1.0, 8.7)	0.9 (0.0, 4.8)	67.0 (57.6, 75.4)	N=115; anthropological-based community surveys	Merryweather-Clarke 1997 (95)
Greenland	4.5 (2.1, 8.4)	0	9.0 (5.4, 13.9)	0	0	86.5 (81.0, 90.9)	N=200; dried blood spots from neonatal screening programs	Merryweather-Clarke 1999 (93)
Greece	2.6 (0.8, 5.9)	0	20.9 (15.5, 27.3)	3.1 (1.1, 6.5)	0	73.4 (66.7, 79.5)	N=196; anthropological-based community surveys and hemoglobinopathy evaluations	Merryweather-Clarke 1997 (95)
Iceland	6.5 (3.7, 10.5)	0.4 (0.0, 2.4)	19.5 (14.6, 25.2)	0.4 (0.0, 2.4)	1.7 (0.5, 4.4)	71.4 (65.1, 77.2)	N=231; dried blood spots from neonatal screening programs	Merryweather-Clarke 1999 (93)
Iceland	10.0 (4.7, 18.1)	0	15.6 (8.8, 24.7)	1.1 (0.0, 6.0)	3.3 (0.7, 9.4)	70.0 (59.4, 79.2)	N=90; blood donors	Merryweather-Clarke 1997 (95)
Ireland	28.4 (20.0, 37.9)	0	24.8 (17.0, 34.0)	3.7 (1.0, 9.1)	3.7 (1.0, 9.1)	39.4 (30.2, 49.3)	N=109; hospital staff controls	Ryan 1998 (84)
Italy	2.2 (0.4, 6.2)	0	20.9 (14.4, 28.6)	1.4 (0.2, 5.1)	0	75.5 (67.5, 82.4)	N=139; control source not specified	Piperno 1998 (85)
Italy	1.1 (0.0, 6.0)	0	20.9 (13.1, 30.7)	2.2 (0.3, 7.7)	0	75.8 (65.7, 84.2)	N=91; community-based surveys of hemoglobinopathies	Merryweather-Clarke 1997 (95)

Northern Ireland	14.8 (11.5, 18.7)	1.2 (0.4, 2.9)	22.8 (18.8, 27.2)	1.5 (0.5, 3.2)	2.5 (1.2, 4.5)	57.2 (52.2, 62.1)	N=404; controls from a bone marrow registry	Murphy 1999 (96)

Table continues

TABLE 2. *HFE* genotype frequencies in the general population (continued)

Study Population	Genotype							
Europe	**C282Y/Wild Frequency (%) (95% CI)**	**C282Y/C282Y Frequency (%) (95% CI)**	**H63D/Wild Frequency (%) (95% CI)**	**H63D/H63D Frequency (%) (95% CI)**	**C282Y/H63D Frequency (%) (95% CI)**	**Wild/Wild Frequency (%) (95% CI)**	**Subjects**	**References**
Norway	12.7 (9.9, 15.9)	0.4 (0.1, 1.4)	18.0 (14.8, 21.7)	1.4 (0.6, 1.4)	2.2 (1.1, 3.9)	65.3 (61.0, 69.5)	N=505; hospital workers residing in greater Oslo area; M=105 and F=400; M median age=37 (range 22-64), F median age=38 (range 20-66)	Distante 1999 (97)
Norway	12.8 (6.9, 21.2)	0	18.1 (10.9, 27.4)	2.1 (0.3, 7.5)	0	67.0 (56.6, 76.4)	N=94; blood donors	Merryweather-Clarke 1997 (95)
Spain	4.5 (2.7, 7.8)	0	32.3 (27.9, 37.1)	4.3 (2.6, 6.7)	1.7 (0.7, 3.4)	57.1 (52.3, 61.9)	N=420; blood donors; M=227 and F=193; mean age=25±8	Sanchez 1998 (98)
Spain	3.8 (0.8, 10.8)	0	32.1 (21.9, 43.6)	9.0 (3.7, 17.6)	2.6 (0.3, 9.0)	52.6 (40.9, 64.0)	N=78; anthropological community-based surveys	Merryweather-Clarke 1997 (95)
Sweden	7.7 (3.6, 14.1)	0	23.1 (15.8, 31.8)	0.9 (0.0, 4.7)	0	68.4 (59.1, 76.7)	N=117; anonymous random samples from healthy subjects	Cardoso 1998 (87)
Turkey	0	0	21.4 (12.5, 32.9)	2.9 (0.3, 9.9)	0	75.7 (64.0, 85.2)	N=70; anthropological community-based surveys and hemoglobinopathy evaluation	Merryweather-Clarke 1997 (95)
United Kingdom	5.9 (2.2, 12.5)	1.0 (0.0, 5.4)	21.8 (14.2, 31.1)	3.0 (0.6, 8.4)	4.0 (1.0, 9.8)	64.3 (54.2, 73.6)	N=101; blood donors from South Wales	Robson 1997 (88)
United Kingdom	7.6 (5.1, 10.8)	0.5 (0.1, 2.0)	20.9 (16.9, 25.4)	0	3.3 (1.7, 5.0)	67.7 (62.2, 72.4)	N=368; family studies of collagen disorders and polycystic kidney disease	Merryweather-Clarke 1997 (95)
USSR (former)	1.9 (0.4, 5.6)	0	18.2 (12.4, 25.2)	1.3 (0.2, 4.6)	0	78.6 (71.2, 84.8)	N=154; anthropological community-based surveys	Merryweather-Clarke 1997 (95)
New Zealand *	11.4 (9.5, 13.4)	0.5 (0.2, 1.1)	22.6 (20.1, 25.2)	2.3 (1.5, 3.3)	1.8 (1.1, 2.8)	61.6 (58.6, 64.5)	N=1,064; volunteers from electoral rolls; M=423 and F=641; mean age=50.2	Burt 1998 (99)
Europe Total	**9.2 (8.5, 10.0)**	**0.4 (0.3, 0.6)**	**21.6 (20.6, 22.6)**	**2.0 (1.6, 2.4)**	**1.8 (1.4, 2.1)**	**65.1 (63.9, 66.3)**	**Europe total number of subjects =6,203**	

Table continues

TABLE 2. *HFE* genotype frequencies in the general population (continued)

Study Population	Genotype							
Africa/ Middle East	**C282Y/Wild Frequency (%) (95% CI)**	**C282Y/C282Y Frequency (%) (95% CI)**	**H63D/Wild Frequency (%) (95% CI)**	**H63D/H63D Frequency (%) (95% CI)**	**C282Y/H63D Frequency (%) (95% CI)**	**Wild/Wild Frequency (%) (95% CI)**	**Subjects**	**References**
Kenya	0	0	2.6 (0.3, 9.0)	0	0	97.4 (91.0, 99.7)	N=78; community-based surveys of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Nigeria	0	0	3.7 (0.8, 10.6)	0	0	96.3 (89.4, 99.2)	N=80; anthropological community-based surveys	Merryweather-Clarke 1997 (95)
Saudi Arabia	0	0	16.9 (10.7, 25.0)	0	0	83.1 (75.0, 89.3)	N=118; community-based surveys of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Senegal	0	0	0	0	0	100 (97.0, 100)	N=130; anthropological-based community surveys	Merryweather-Clarke 1997 (95)
South Africa	0.5 (0.0, 2.8)	0	0	0	0	99.5 (97.2, 100)	N=200; African controls	De Villiers 1999 (100)
Zambia	0	0	1.3 (0.0, 7.1)	0	0	98.7 (92.9, 96.6)	N=76; neonatal surveys of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Africa/M. East Total	**0.2 (0.0, 1.2)**	**0**	**5.4 (3.6, 7.8)**	**0**	**0**	**94.4 (92.0, 96.3)**	**Africa/Middle East total number of subjects=483**	

Table continues

TABLE 2. *HFE* genotype frequencies in the general population (continued)

	Genotype							
Indian subcont-inent	**C282Y/Wild**	**C282Y/C282Y**	**H63D/Wild**	**H63D/H63D**	**C282Y/H63D**	**Wild/Wild**	**Subjects**	**References**
India/ Pakistan	0.9 (0.0, 5.1)	0	15.1 (8.9, 23.4)	0	0	84.0 (75.6, 90.4)	N=106; individuals referred for diagnosis of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Sri Lanka	0	0	16.5 (10.1, 24.8)	0.9 (0.0, 5.0)	0	82.6 (74.1, 89.2)	N=109; individuals referred for diagnosis of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Indian Subcont-inent Total	**0.5 (0.0, 2.6)**	**0**	**15.8 (11.2, 21.4)**	**0.5 (0.0, 2.6)**	**0**	**83.3 (77.6, 88.0)**	**Indian subcontinent total number of subjects=215**	
Asia								
Chinese (Hong Kong)	0	0	5.5 (1.5, 13.6)	0	0	94.4 (86.4, 98.5)	N=72; hemoglobinopathy evaluation and anthropological community-based surveys	Merryweather-Clarke 1997 (95)
Indonesian	0	0	5.5 (1.8, 12.5)	0	0	94.4 (87.5, 98.2)	N=90; anthropological community-based surveys	Merryweather-Clarke 1997 (95)
Japan	0	0	2.0 (0.7, 4.6)	0	0	98.0 (95.4, 99.4)	N=252; healthy volunteers	Sohda 1999 (101)
Taiwan (Aboriginal)	0	0	0	0	0	100 (95.5, 100)	N=80; anthropological community-based surveys	Merryweather-Clarke 1997 (95)
Asian Total	**0**	**0**	**2.8 (1.6, 4.7)**	**0**	**0**	**97.2 (95.3, 98.4)**	**Asia total number of subjects=494**	
Australasia								
Australia (Aborigonal)	0	0	0	0	0	100 (94.5, 100)	N=93; anthropological-based community surveys	Merryweather-Clarke 1997 (95)
Australia (Vanuatuan)	0	0	1.1 (0.0, 6.0)	0	0	98.9 (94.0, 100)	N=90; community-based surveys of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Papua New Guinea	0	0	0	0	0	100 (97.4, 100)	N=139; community-based malarial survey	Merryweather-Clarke 1997 (95)
Australasia Total	**0**	**0**	**0.3 (0.0, 1.7)**	**0**	**0**	**99.7 (98.3, 100)**	**Australasia total number of subjects=322**	

Table continues

TABLE 2. *HFE* genotype frequencies in the general population (continued)

Study Population	Genotype							
Americas	**C282Y/Wild Frequency (%) (95% CI)**	**C282Y/C282Y Frequency (%) (95% CI)**	**H63D/Wild Frequency (%) (95% CI)**	**H63D/H63D Frequency (%) (95% CI)**	**C282Y/H63D Frequency (%) (95% CI)**	**Wild/Wild Frequency (%) (95% CI)**	**Subjects**	**References**
Jamaica	2.2 (0.3, 7.8)	0	4.4 (1.2, 11.0)	0	0	93.3 (86.1, 97.5)	N=90; community-based surveys of hemoglobinopathies	Merryweather-Clarke 1997 (95)
Mexico	0	0	13.0 (5.4, 24.9)	0	0	87.0 (75.1, 94.6)	N=54; anthropological community-based surveys	Merryweather-Clarke 1997 (95)
United States	6.4 (3.1, 11.5)	0	32.9 (25.6, 40.9)	0	0	60.6 (52.5, 68.4)	N=155; 64 whites of the grandparental generation in the CEPH collection and 92 random white individuals	Feder 1996 (4)
United States	13.1 (8.8, 18.5)	0	21.8 (16.4, 28.1)	3.4 (1.4, 6.9)	1.0 (0.1, 3.5)	54.4 (47.3, 61.3)	N=193; persons of European origin	Beutler 1996 (90)
United States (Alabama)	10.6 (6.0, 16.8)	0.7 (0.0, 3.9)	19.7 (13.5, 27.2)	2.8 (0.8, 7.1)	3.5 (1.2, 8.0)	62.7 (54.2, 70.6)	N=142; randomly selected white controls from the Birmingham area as cases	Barton 1997 (20)
United States (Connecticut)	1.8 (0.1, 9.6)	0	3.6 (0.4, 12.3)	0	0	94.6 (85.1, 98.9)	N=56; randomly selected African American patients from Hartford Hospital	Marshall 1999 (102)
United States (Connecticut)	8.0 (3.5, 15.2)	1.0 (0.0, 5.4)	24.0 (16.0, 33.6)	4.0 (1.1, 9.9)	0	63.0 (52.8, 72.4)	N=100; randomly selected white patients from Hartford Hospital	Marshall 1999 (102)
United States (Connecticut)	3.0 (0.6, 8.5)	0	15.0 (8.6, 23.5)	1.0 (0.0, 5.4)	1.0 (0.0, 5.4)	80.0 (70.8, 87.3)	N=100; randomly selected Hispanic patients from Hartford Hospital	Marshall 1999 (102)
United States (Maine)	9.7 (7.9, 11.7)	0.7 (0.3, 1.4)	24.6 (21.9, 27.4)	1.7 (1.0, 2.7)	2.2 (1.4, 3.3)	61.1 (58.0, 64.2)	N=1,001; cohort of couples undergoing prenatal screening for cystic fibrosis	Bradley 1998 (103)
United States (Missouri)	8.9 (7.5, 10.5)	0.4 (0.2, 0.9)	23.9 (21.8, 26.2)	3.4 (2.6, 4.5)	2.5 (1.7, 3.3)	60.9 (58.3, 63.4)	N=1,450; health maintenance organization employee volunteers; 3 C282Y/C282Y, 1 H63D/H63D and 1 C282Y/H63D had HH with iron overload; M=288 and F=1,365; mean age=41±11	McDonnell 1999 (27)
United States (New Jersey)	11.4 (8.5, 14.9)	1.0 (0.3, 2.5)	20.9 (17.1, 25.2)	2.9 (1.5, 5.0)	3.2 (1.7, 5.3)	60.6 (55.7, 65.3)	N=411; blood donors; 79 donors with 4 grandparents (GP) from Channel Islands, 131 with ≥ 1 GP from Jersey, 173 with 4 GP from UK or Ireland, 18 with ≥ 1 GP from UK, 10 with other ethnic origin	Merryweather-Clarke 1997 (95)
Americas Total	**9.0 (8.1, 10.0)**	**0.5 (0.3, 0.8)**	**22.8 (21.5, 24.2)**	**2.5 (2.1, 3.1)**	**2.1 (1.6, 2.6)**	**63.1 (61.5, 64.6)**	**Americas total number of subjects=3,752**	
Global Total	**7.8 (7.4, 8.3)**	**0.4 (0.3, 0.5)**	**19.4 (18.7, 20.2)**	**1.9 (1.6, 2.1)**	**1.6 (1.4, 1.9)**	**68.9 (68.0, 69.7)**	**Global total number of subjects=11,668**	

N=number, M=males, F=females

* Study is included in European because 1,021 of the volunteers were of European origin from Christchurch, New Zealand; 23 (2.2%) Maori; 8 (0.8%) Pacific Islander, and 12 (1.1%) other.

References

1. Simon M, Bourel M, Fauchet R, et al. HLA and "non-immunological" disease: idiopathic haemochromatosis [letter]. Lancet 1976;2:973-4.
2. Cartwright GE, Skolnick M, Amos DB, et al. Inheritance of hemochromatosis: linkage to HLA. Trans Assoc Am Physicians 1978;91:273-81.
3. Dadone MM, Kushner JP, Edwards CQ, et al. Hereditary hemochromatosis. Analysis of laboratory expression of the disease by genotype in 18 pedigrees. Am J Clin Pathol 1982;78:196-207.
4. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399-408.
5. Bodmer JG, Marsh SG, Albert ED, et al. Nomenclature for factors of the HLA system, 1996. Vox Sang 1997;73:105-30.
6. Rhodes DA, Trowsdale J. Alternate splice variants of the hemochromatosis gene HFE. Immunogenetics 1999;49:357-9.
7. Feder JN, Penny DM, Irrinki A, et al. The hemochromatosis gene product complexes with the transferrin receptor and lowers its affinity for ligand binding. Proc Natl Acad Sci U S A 1998;95:1472-7.
8. Lebron JA, Bennet MJ, Vaughn DE, et al. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell 1998;93:111-23.
9. Waheed A, Parkkila S, Saarnio J, et al. Association of HFE protein with transferrin receptor in crypt enterocytes of human duodenum. Proc Natl Acad Sci U S A 1999;96:1579-84.

10. Feder JN, Tsuchihashi Z, Irrinki A, et al. The hemochromatosis founder mutation in HLA-H disrupts beta2- microglobulin interaction and cell surface expression. J Biol Chem 1997;272:14025-8.

11. Parkilla S, Waheed A, Britton RS, et al. Immunohistochemistry of HLA-H, the protein defective in patients with hemochromatosis, reveals unique pattern of expression in hereditary hemochromatosis. Proc Natl Acad Sci USA 1997;94:2534-39.

12. Zhou XY, Tomatsu S, Fleming RE, et al. HFE gene knockout produces mouse model of hereditary hemochromatosis. Proc Natl Acad Sci U S A 1998;95:2492-7.

13. Pointon JJ, Wallace D, Merryweather-Clarke AT, et al. Uncommon mutations and polymorphisms in the hemochromatosis gene. Genetic Testing 2000;4:151-61.

14. Mura C, Raguenes O. HFE mutations analysis in 711 hemochromatosis probands: Evidence for S65C implication in mild form of hemochromatosis. Blood 1999;93:2502-5.

15. Jeffrey GP, Chakrabarti S, Hegele RA, et al. Polymorphism in intron 4 of HFE may cause overestimation of C282Y homozygote prevalence in haemochromatosis. Nat Genet 1999;22:325-6.

16. Sommerville MJ, Sprysak KA, Hicks M, et al. An HFE intronic variant promotes misdiagnosis of hereditary hemochromatosis [letter]. Am J Hum Genet 1999;65:924-6.

17. EASL International consensus conference on haemochromatosis. J Hepat 2000;33:485-504.

18. Noll WW, Belloni DR, Stenzel TT, et al. Polymorphism in intron 4 of HFE does not compromise haemochromatosis mutation results [letter; comment]. Nat Genet 1999;23:271-2.

19. Elsea SH, Leykam V. HFE polymorphism and accurate diagnosis of C282Y hereditary hemochromatosis carriers [letter]. Blood 2000;95:2453-5.

20. Barton JC, Shih WW, Sawada-Hirai R, et al. Genetic and clinical description of hemochromatosis probands and heterozygotes: evidence that multiple genes linked to the major histocompatibility complex are responsible for hemochromatosis. Blood Cells Mol Dis 1997;23:135-45.

21. Moirand R, Jouanolle AM, Brissot P, et al. Phenotypic expression of HFE mutations: a French study of 1110 unrelated iron-overloaded patients and relatives. Gastroenterology 1999;116:372-7.

22. Brissot P, Moirand R, Jouanolle AM, et al. A genotypic study of 217 unrelated probands diagnosed as "genetic hemochromatosis" on "classical" phenotypic criteria. J Hepatol 1999;30:588-93.

23. Tay G, Leelayuwat C, Chorney M, et al. The MHC contains multiple genes potentially relevant to hemochromatosis. Immunogenetics 1997;45:336-40.

24. Whitfield JB, Cullen LM, Jazwinska EC, et al. Effects of HFE C282Y and H63D polymorphisms and polygenic background on iron stores in a large community sample of twins. Am J Hum Genet 2000;66:1246-58.

25. Bradley LA, Haddow JE, Palomaki GE. Population screening for haemochromatosis: expectations based on a study of relatives of symptomatic probands. J Med Screen 1996;3:171-7.

26. Phatak PD, Sham RL, Raubertas RF, et al. Prevalence of hereditary hemochromatosis in 16,031 primary care patients. Ann Intern Med 1998;129:954-61.

27. McDonnell SM, Hover A, Gloe D, et al. Population-based screening for hemochromatosis using phenotypic and DNA testing among employees of health maintenance organizations in Springfield, Missouri. Amer J Med 1999;107:30-7.

28. Camaschella C, Fargion S, Sampietro M, et al. Inherited HFE-unrelated hemochromatosis in Italian families. Hepatology 1999;29:1563-4.

29. Pietrangelo A, Montosi G, Totaro A, et al. Hereditary hemochromatosis in adults without pathogenic mutations in the hemochromatosis gene. N Engl J Med 1999;341:725-32.

30. Camaschella C, Roetto A, Cali A, et al. The gene TFR2 is mutated in a new type of haemochromatosis mapping to 7q22. Nat Genet 2000;25:14-5.

31. Roetto A, Totaro A, Cazzola M, et al. Juvenile hemochromatosis locus maps to chromosome 1q. Am J Hum Genet 1999;64:1388-93.

32. Bothwell TH, MacPhail AP. Hereditary hemochromatosis: etiologic, pathologic, and clinical aspects. Semin Hematol 1998;35:55-71.

33. Adams PC, Deugnier Y, Moirand R, et al. The relationship between iron overload, clinical symptoms, and age in 410 patients with genetic hemochromatosis. Hepatology 1997;25:162-6.

34. Niederau C, Fischer R, Purschel A, et al. Long-term survival in patients with hereditary hemochromatosis. Gastroenterology 1996;110:1107-19.

35. Adams PC, Valberg LS. Evolving expression of hereditary hemochromatosis. Semin Liver Dis 1996;16:47-54.

36. Adams PC, Speechley M, Kertesz AE. Long-term survival analysis in hereditary hemochromatosis. Gastroenterology 1991;101:368-72.

37. Moirand R, Adams PC, Bicheler V, et al. Clinical features of genetic hemochromatosis in women compared with men. Ann Intern Med 1997;127:105-10.

38. Niederau C, Fischer R, Sonnenberg A, et al. Survival and causes of death in cirrhotic and in noncirrhotic patients with primary hemochromatosis. N Engl J Med 1985;313:1256-62.

39. Fargion S, Mandelli C, Piperno A, et al. Survival and prognostic factors in 212 Italian patients with genetic hemochromatosis. Hepatology 1992;15:655-9.

40. Cogswell ME, McDonnell SM, Khoury MJ, et al. Iron overload, public health, and genetics: evaluating the evidence for hemochromatosis screening. Ann Intern Med 1998;129:971-9.

41. Witte DL, Crosby WH, Edwards CQ, et al. Practice guideline development task force of the College of American Pathologists. Hereditary hemochromatosis. Clin Chim Acta 1996;245:139-200.

42. Adams PC. Hemochromatosis: new insights in pathogenesis and diagnosis following the discovery of the gene. Crit Rev Clin Lab Sci 1998;35:239-73.

43. Edwards CQ, Dadone MM, Skolnick MH, et al. Hereditary haemochromatosis. Clin Haematol 1982;11:411-35.

44. Finch SC, Finch CA. Idiopathic hemochromatosis, an iron storage disease. A. Iron metabolism in hemochromatosis. Medicine 1955;34:381-430.

45. Yip R. Iron deficiency. Bull World Health Organ 1998;76:121-3.

46. Powell LW, Bassett ML. Haemochromatosis: diagnosis and management after the cloning of the HFE gene. Aust N Z J Med 1998;28:159-63.

47. Niederau C, Niederau CM, Lange S, et al. Screening for hemochromatosis and iron deficiency in employees and primary care patients in Western Germany. Ann Intern Med 1998;128:337-45.

48. Crawford DH, Jazwinska EC, Cullen LM, et al. Expression of HLA-linked hemochromatosis in subjects homozygous or heterozygous for the C282Y mutation. Gastroenterology 1998;114:1003-8.

49. Barton JC, McDonnell SM, Adams PC, et al. Management of hemochromatosis. Hemochromatosis Management Working Group. Ann Intern Med 1998;129:932-9.

50. Bomford A, Williams R. Long term results of venesection therapy in idiopathic haemochromatosis. Q J Med 1976;45:611-23.

51. Powell LW, Kerr JF. Reversal of "cirrhosis" in idiopathic haemochromatosis following long-term intensive venesection therapy. Australas Ann Med 1970;19:54-7.

52. Crosby WH. A history of phlebotomy therapy for hemochromatosis. Am J Med Sci 1991;301:28-31.

53. Yang Q, McDonnell SM, Khoury MJ, et al. Hemochromatosis-associated mortality in the United States from 1979 to 1992: an analysis of multiple-cause mortality data. Ann Intern Med 1998;129:946-53.

54. Burke W, Imperatore G, McDonnell SM, et al. Contribution of different genotypes to iron overload disease: a pooled analysis. Genetics Med (in press).

55. Levy JE, Montross LK, Andrews NC. Genes that modify the hemochromatosis phenotype in mice. J Clin Invest 2000;105:1209-16.

56. Burke W, Press N, McDonnell SM. Hemochromatosis: genetics helps to define a multifactorial disease. Clin Genet 1998;54:1-9.

57. Bonkovsky HL, Poh-Fitzpatrick M, Pimstone N, et al. Porphyria cutanea tarda, hepatitis C, and HFE gene mutations in North America. Hepatology 1998;27:1661-9.

58. Conte D, Manachino D, Colli A, et al. Prevalence of genetic hemochromatosis in a cohort of Italian patients with diabetes mellitus. Ann Intern Med 1998;128:370-3.

59. Frayling T, Ellard S, Grove J, et al. C282Y mutation in HFE (haemochromatosis) gene and type 2 diabetes [letter]. Lancet 1998;351:1933-4.

60. Dykes A, Volker G, Firkin F. Aetiology of severe iron overload in a family with hereditary haemolytic anemia. Br J Haematol 1998;102:1179-81.

61. McDonnell SM, Phatak PD, Felitti V, et al. Screening for hemochromatosis in primary care settings. Ann Intern Med 1998;129:962-70.

62. Adams PC, Gregor JC, Kertesz AE, et al. Screening blood donors for hereditary hemochromatosis: decision analysis model based on a 30-year database. Gastroenterology 1995;109:177-88.

63. Bradley LA, Haddow JE, Palomaki GE. Population screening for haemochromatosis: a unifying analysis of published intervention trials. J Med Screen 1996;3:178-84.

64. Edwards CQ, Griffen LM, Ajioka RS, et al. Screening for hemochromatosis: phenotype versus genotype. Semin Hematol 1998;35:72-6.

65. Bell H, Skinningsrud A, Raknerud N, et al. Serum ferritin and transferrin saturation in patients with chronic alcoholic and non-alcoholic liver diseases. J Intern Med 1994;236:315-22.

66. George DK, Evans RM, Crofton RW, et al. Testing for haemochromatosis in the diabetic clinic. Ann Clin Biochem 1995;32:521-6.

67. O'Brien T, Barrett B, Murray DM, et al. Usefulness of biochemical screening of diabetic patients for hemochromatosis. Diabetes Care 1990;13:532-4.

68. McDonnell SM, Witte D. Hereditary hemochromatosis: preventing chronic effects of this underdiagnosed disorder. Postgrad Med 1997;102:83-5, 8-91, 4.

69. Adams PC, Chakrabarti S. Genotypic/phenotypic correlations in genetic hemochromatosis: evolution of diagnostic criteria. Gastroenterology 1998;114:319-23.

70. Bacon BR. Diagnosis and management of hemochromatosis. Gastroenterology 1997;113:995-9.

71. Willis G, Wimperis JZ, Smith KC, et al. Haemochromatosis gene C282Y homozygotes in an elderly male population [letter]. Lancet 1999;354:221-2.

72. Resident population estimates of the United States by sex, race and Hispanic Origin: April 1, 1990 to November 1, 1999: U.S. Census Bureau, 1999: http//www.census.gov/population/estimates/nation/intfile3-1.txt.

73. Burke W, Thomson E, Khoury MJ, et al. Hereditary hemochromatosis: gene discovery and its implications for population-based screening. JAMA 1998;280:172-8.

74. Haddow JE, Bradley LA. Hereditary haemochromatosis: to screen or not. Conditions for screening are not yet fulfilled [editorial]. BMJ 1999;319:531-2.

75. Sampson MJ, Williams T, Heyburn PJ, et al. Prevalence of HFE (hemochromatosis gene) mutations in unselected male patients with type 2 diabetes. J Lab Clin Med 2000;135:170-3.

76. Alper JS, Geller LN, Barash CI, et al. Genetic discrimination and screening for hemochromatosis. J Public Health Policy 1994;15:345-8.

77. Balan V, Baldus W, Fairbanks V, et al. Screening for hemochromatosis: a cost-effectiveness study based on 12,258 patients. Gastroenterology 1994;107:453-9.

78. Phatak PD, Guzman G, Woll JE, et al. Cost-effectiveness of screening for hereditary hemochromatosis. Arch Intern Med 1994;154:769-76.

79. Adams PC. Implications of genotyping of spouses to limit investigation of children in genetic hemochromatosis. Clin Genet 1998;53:176-8.

80. El-Serag HB, Inadomi JM, Kowdley KV. Screening for hereditary hemochromatosis in siblings and children of affected patients. A cost-effectiveness analysis. Ann Intern Med 2000;132:261-9.

81. Aguilar Martinez P, Biron C, Blanc F, et al. Compound heterozygotes for hemochromatosis gene mutations: may they help to understand the pathophysiology of the disease? Blood Cells Mol Dis 1997;23:269-76.

82. Borot N, Roth M, Malfroy L, et al. Mutations in the MHC class I-like candidate gene for hemochromatosis in French patients. Immunogenetics 1997;45:320-4.

83. Gottschalk R, Seidl C, Loffler T, et al. HFE codon 63/282 (H63D/C282Y) dimorphism in German patients with genetic hemochromatosis. Tissue Antigens 1998;51:270-5.

84. Ryan E, O'Keane C, Crowe J. Hemochromatosis in Ireland and HFE. Blood Cells Mol Dis 1998;24:428-32.

85. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy. Gastroenterology 1998;114:996-1002.

86. Miedzybrodzka Z, Loughlin S, Baty D, et al. Haemochromatosis mutations in North-East Scotland. Br J Haematol 1999;106:385-7.

87. Cardoso EM, Stal P, Hagen K, et al. HFE mutations in patients with hereditary haemochromatosis in Sweden. J Intern Med 1998;243:203-8.

88. Robson KJ, Worwood M, Shearman JD. A simple genetic test identifies 90% of UK patients with haemochromatosis. The UK Haemochromatosis Consortium. Gut 1997;41:841-4.

89. Sham RL, Ou CY, Cappuccio J, et al. Correlation between genotype and phenotype in hereditary hemochromatosis: analysis of 61 cases. Blood Cells Mol Dis 1997;23:314-20.

90. Beutler E, Gelbart T, West C, et al. Mutation analysis in hereditary hemochromatosis. Blood Cells Mol Dis 1996;22:187-94.

91. Jazwinska EC, Cullen LM, Busfield F, et al. Haemochromatosis and HLA-H [letter]. Nat Genet 1996;14:249-51.

92. Steffensen R, Varming K, Jersild C. Determination of gene frequencies for two common haemochromatosis mutations in the Danish population by a novel polymerase chain reaction with sequence-specific primers. Tissue Antigens 1998;52:230-5.

93. Merryweather-Clarke AT, Simonsen H, Shearman JD, et al. A retrospective anonymous pilot study in screening newborns for HFE mutations in Scandinavian populations. Hum Mutat 1999;13:154-9.

94. Jezequel P, Bargain M, Lellouche F, et al. Allele frequencies of hereditary hemochromatosis gene mutations in a local population of west Brittany. Hum Genet 1998;102:332-3.

95. Merryweather-Clarke AT, Pointon JJ, Shearman JD, et al. Global prevalence of putative haemochromatosis mutations. J Med Genet 1997;34:275-8.

96. Murphy S, Curran MD, McDougall N, et al. High incidence of the Cys282Tyr mutation in the HFE gene in the Irish population- implications for haemochromatosis. Tissue Antigens 1998;52:484-8.

97. Distante S, Berg JP, Lande K, et al. High Prevalence of the hemochromatosis-associated Cys282Tyr HFE gene mutation in a healthy Norwegian population in the city of Oslo, and its phenotypic expression. Scand J Gastroenterol 1999;5:529-34.

98. Sanchez M, Brugera M, Bosch J, et al. Prevalence of the Cys282Tyr and His63Asp HFE gene mutations in Spanish patients with hereditary hemochromatosis and in controls. J Hepatol 1998;29:725-8.

99. Burt MJ, George PM, Upton JD, et al. The significance of haemochromatosis gene mutations in the general population: implications for screening. Gut 1998;43:830-6.

100. de Villiers JN, Hillerman R, de Jong G, et al. High prevalence of the Cys282Tyr HFE mutation facilitates an improved diagnostic service for hereditary haemochromatosis in South Africa. S Afr Med J 1999;89:279-82.

101. Sohda T, Yanai J, Soejima H, et al. Frequencies in the Japanese population of HFE gene mutations. Biochem Gen 1999;37:63-8.

102. Marshall DS, Linfert DR, Tsongalis GJ. Prevalence of the C282Y and H63D polymorphisms in a multi-ethnic control population. Int J Mol Med 1999;4:389-93.

103. Bradley LA, Johnson DD, Palomaki GE, et al. Hereditary hemochromatosis mutation frequencies in the general population. J Med Screen 1998;5:34-6.